All-polymer chip system for magnetic bead-based solid phase extraction

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ALL-POLYMER CHIP SYSTEM FOR MAGNETIC BEAD-BASED SOLID PHASE EXTRACTION

INTRODUCTION

Paramagnetic particles or magnetic beads (MBs) are commonly used as the solid phase matrix for magnetic bead-based solid phase extraction (SPE). A variant of MB-based SPE exists, where an immiscible phase is used as a filtering step in order to circumvent the washing steps otherwise needed to perform a successful extraction [1-3]. The principle of the technology is presented in the sketch below.

In this study we present an injection moulded cyclic olefin copolymer (COC) planar chip system that has been bonded together using ultrasonic welding – both techniques that can be readily applied in mass production and it is what sets this system apart from ones previous published. The chip is fitted with geometric capillary micro valves for MB-based SPE using the immiscible phase filtration approach. See figure 1 for a photograph of the chip.

We

- Characterise the chip in regard to carry-over volume and further investigate the influence of surfactants on the efficacy of the system.
- Present initial performance results, by detecting respiratory syncytial virus (RSV) in a mucus sample.

[1] K. Sur, S.M. McFall, E.T. Yeh, S.R. Jangam, M. a Hayden, S.D. Stroupe, et al., Immiscible phase nucleic acid purification eliminates PCR inhibitors with a single pass of paramagnetic particles through a hydrophobic liquid., J. Mol. Diagn. 12 (2010) 620–8. doi:10.2353/jmoldx.2010.090190.

[2] S.M. Berry, E.T. Alarid, D.J. Beebe, One-step purification of nucleic acid for gene expression analysis via Immiscible Filtration Assisted by Surface Tension (IFAST)., Lab Chip. 11 (2011) 1747–53. doi:10.1039/c1lc00004g.

[3] R.C. den Dulk, K. a Schmidt, G. Sabatté, S. Liébana, M.W.J. Prins, Magneto-capillary valve for integrated purification and enrichment of nucleic acids and proteins., Lab Chip. (2012) 106–118. doi:10.1039/c2lc40929a.

PRINCIPLE OF OPERATION

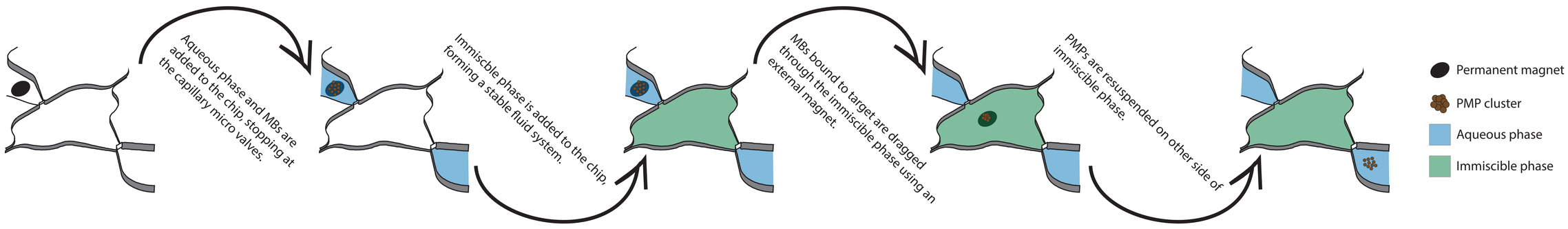
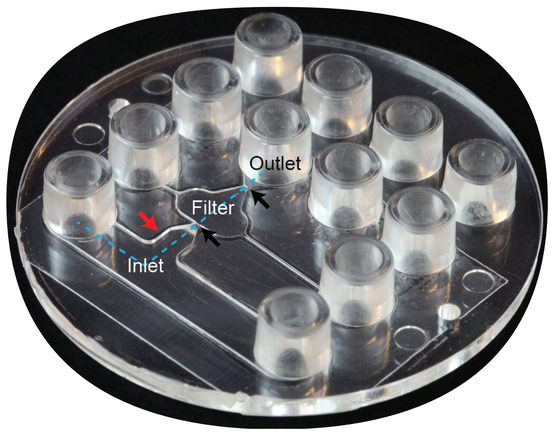


FIGURE 1



Photograph of a welded chip with the different compartments highlighted. The red arrow indicates the welding seam following the channel circumference. The black arrows indicate the positions of the geometric capillary micro valves. The blue dotted line shows the pathway of the magnetic bead cluster. Ø = 50 mm.

RESULTS

The chip was performance tested in regard to volume carry-over and ability to detect RSV. The chip was tested with various surfactants and the carry-over volume was quantified.

Figure 2 shows the determination of volume carry-over vs. amount of MyOne SILANE magnetic beads for pure water and a typical XNA lysis/binding buffer.

We find that the volume carry-over;

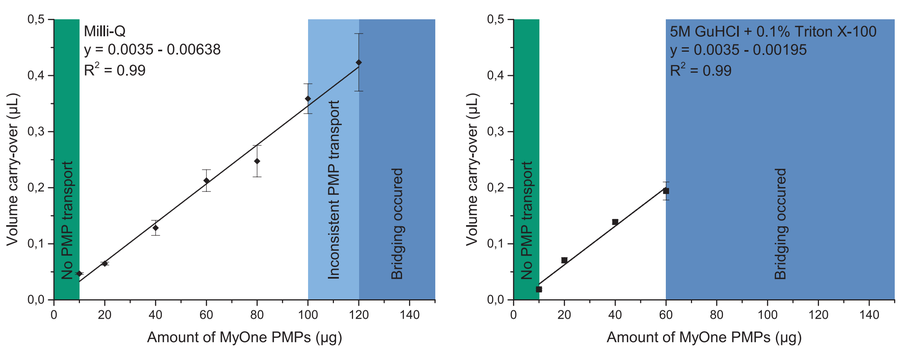
- is proportional to the amount of beads through a linear correlation.
- is the same for Milli-Q water and the typical lysis/binding buffer.

Figure 3 shows initial results on RNA extraction, comparing the on-chip assay with an off-chip reference.

We find that;

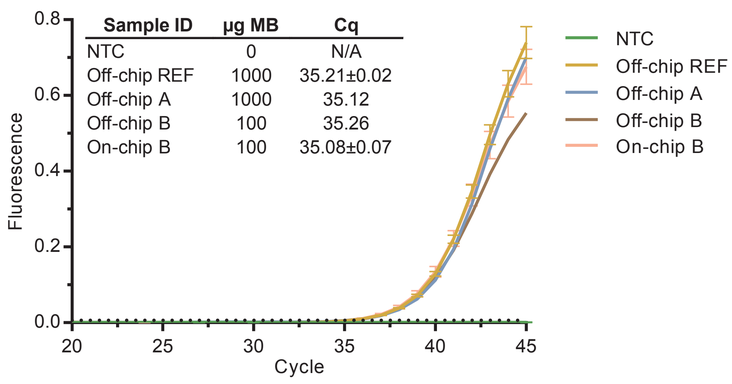
- Reducing the MB amount to one compatible with the chip had no effect on Cq.
- The on-chip extraction performed on par with the off-chip extraction.

FIGURE 2



Volume carry-over for various amounts of MyOne SILANE MBs. The left part shows the carry-over of Milli-Q water whilst the right part shows the carry-over of a 5M guanidine hydrochloride solution with 0.1% Triton x-100. A good correlation was found in both cases with an average carry-over of 0.0035 µl/µg. It was not possible to transfer less than 10 µg of MBs. For Milli-Q water, MB transfer became problematic above 100 µg and bridging occurred above 120 µg. For the surfactant containing solution bridging occurred already at loads of 60 µg PMPs.

FIGURE 3



Detection of RSV (RNA) in a mucus sample. The graph shows the amplification curves of the investigated samples. All template-containing samples performed equally well. Embedded table shows the actual Cq (quantification cycle) values. Dotted line represents the Cq threshold, n = 2, 2, 1, 1, and, 2, respectively. Error bars represent SD and NTC is the no template control.

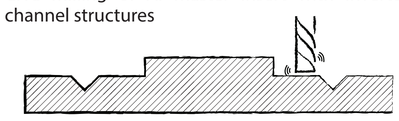
CHIP FABRICATION

The chip consists of two cyclic olefin copolymer parts of the grade TOPAS 5013; An injection moulded main part and a 0.152 mm extruded film.

The fabrication process is as follows:

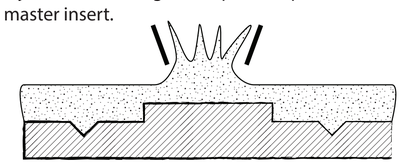
(A) MILLING OF MOULD INSERT

CNC milling of Al master insert with inverted channel structures



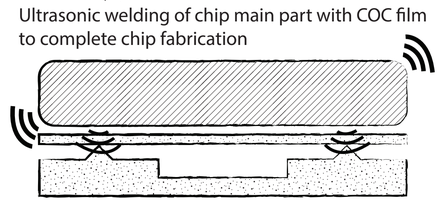
(B) INJECTION MOULDING

Injection moulding of chip main part based on master insert.



(C) ULTRASONIC WELDING

Ultrasonic welding of chip main part with COC film to complete chip fabrication



(D) FINISHED CHIP

METHODS

The assembled chip is disc shaped and features a Luer-Slip layout with an inlet channel, oil-containing "filtration" channel and an outlet chamber, all interconnected by geometric capillary micro valves, see Figure 1. The chip was mounted in a setup with a movable magnet situated under the inlet Luer fitting.

VOLUME CARRY-OVER QUANTIFICATION

A fluorophore containing solution was added to the inlet channel and a blank solution to the outlet chamber. FC40 oil was then added to the middle channel to complete the loading. Various volumes of MBs was then added to the inlet and transferred from inlet to outlet by moving the magnet. The MBs were resuspended, removed and the outlet volume was transferred to a microtiter plate. The carry-over volume was estimated by analysing the dye content of the wells. This concentration could then be calculated into a volume by correlating with the dye concentration of the inlet and the respective volumes of the inlet and outlet.

OFF-CHIP EXTRACTION PROTOCOL

100 µl Lysis/binding buffer (4.55 M GuHCl (AppliChem GmbH, Germany) + 0.1(V/V)% Triton X-100 (Sigma-Aldrich) in citrate buffer, pH 3.9), 100 or 1000 µg MyOne SILANE beads (Life technologies, CA, USA) and 100 µl mucus sample (dissolved 1:1 in salt water) was added to a 1.5 ml tube, mixed by pipetting, and left to incubate at RT for 10 min. The beads were then washed thrice by moving the beads by PickPen (Bio-Nobile, Finland) to new 1.5 ml tubes containing WBI, WBII, and WBIII from the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche, Switzerland). Finally the RNA was eluded in 10 mM TE buffer at 70 °C for 5 min and quantified by RT-qPCR using the OneStep RT-PCR kit (Qiagen, Germany) with EvaGreen dye (Biotium Inc., CA, USA) in a Chromo4 real-time detector (Bio-rad, CA, USA). REF sample was extracted using the complete Roche kit.

ON-CHIP EXTRACTION PROTOCOL

100 µl Lysis/binding buffer, 100 µg MyOne SILANE beads and 100 µl mucus sample (dissolved 1:1 in salt water) was added to a 1.5 ml tube, mixed by pipetting, and left to incubate at RT for 10 min. The solution was then transferred to the "inlet" chamber of the chip, which had been pre-loaded with 50 µl 10 mM TE buffer in the "outlet" chamber. 50 µl FC40 oil was then added to the chip to complete the loading. The beads where then transferred to the "outlet" chamber using the external magnet and the contents of the elution chamber were transferred to a 1.5 ml tube and eluded at 70 °C for 5 min. RNA was quantified as above.

CONCLUSION/OUTLOOK

We have demonstrated a mass-producible all-polymer chip created for Mb-based solid phase extraction via immiscible phase filtration. It shows a low volume carry-over and is capable of extracting viral RNA from a mucus sample. Future studies include a more thorough investigation of RNA extraction and a possible switch in polymer type for chip manufacturing. The COC used here is not optimal for a system where you wish to employ surfactants. A polymer with a higher surface energy would be more beneficial.

HAVE A LOOK AT THE CHIP

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